THE COUNCIL FOR TOBACCO RESEARCH–U.S.A., INC.

110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8885

Application for Research Grant
(Use extra pages as needed)

Date: 11/19/74

1. Principal Investigator (give title and degrees):

G. J. Gleich, M.D.
Consultant in Medicine, Mayo Clinic and Mayo Foundation
Associate Professor of Internal Medicine and Microbiology, Mayo Medical School

2. Institution & address:

Mayo Foundation
200 First Street Southwest
Rochester, Minnesota 55901

3. Department(s) where research will be done or collaboration provided:

Department of Medicine and Allergic Disease Research Laboratory

4. Short title of study:

Hypersensitivity to Antigens from Tobacco As a Factor in the Pathogenesis of Chronic Bronchitis

5. Proposed starting date: July 1, 1975

6. Estimated time to complete: 12 months

7. Brief description of specific research aims:

We will determine whether antigens are present in tobacco and tobacco smoke when these materials are used to immunize rabbits. We will also determine whether antigens present in tobacco or tobacco smoke are able to stimulate immune responses in humans with chronic bronchitis.

8. Brief statement of working hypothesis:

Although chronic bronchitis and pulmonary emphysema are associated with chronic exposure to tobacco smoke, the mechanism by which these diseases are caused remains obscure. The effects of smoking tobacco likely include 1) a primary irritant effect on the bronchi themselves, possibly mediated by small molecular weight materials derived from the smoke, 2) the presence in tobacco smoke of materials which alter the function of host resistance mechanisms, particularly of alveolar macrophages (1), and 3) a deficiency in the flow of mucous such that the clearing mechanism of the lung is interfered with and the organ is thus prone to colonization by bacteria. These are the possibilities which have been considered most strongly in the pathogenesis of chronic bronchitis (2-6). Another possibility is that tobacco smoke may contain materials which are capable of functioning as antigens in the lung and which incite a chronic immunologically mediated inflammatory reaction. Positive immediate wheal and flare reactions to tobacco and tobacco smoke extracts (7) and the demonstration of what appears to be IgE antibodies by passive transfer (Prausnitz-Kustner method) (8,9) suggest that in some individuals there may be an immune response to constituents of tobacco or tobacco smoke (10).

9. Details of experimental design and procedures (append extra pages as necessary)

RESEARCH PLAN

A. Analysis of Extracts of Tobacco

1. The first step of the experiments will be to prepare an extract of commercial cigarette tobacco. This will be accomplished by stirring cigarette tobacco in buffered saline at room temperature for twenty-four hours. Toluene will be added for bacteriostasis. The mixture will be centrifuged and the supernatant decanted and saved. The supernatant will be analyzed for protein content by precipitation with trichloracetic acid, solubilization in dilute alkali and biuret testing. Electrophoresis of the solubilized precipitate on polyacrylamide gel at acid and alkaline pH and by isoelectrofocusing will be performed and finally the supernatant will be fractionated by gel filtration and ion exchange chromatography to isolate any major antigens.

2. A portion of the tobacco extract will also be mixed with Freund's complete adjuvant and injected into rabbits. The animals will be bled at three weeks and the serum tested for antibodies to tobacco extract by immuno-precipitation. If no antibodies are detected or if they are present only in low titer, the rabbits will be re-injected with tobacco extract plus Fruend's adjuvant and test bleedings carried out at appropriate intervals. When a satisfactory titer of precipitating antibody is present in the serum, a larger sample of serum will be collected and frozen.

3. Attempts will next be made to characterize the antigen or antigens present in tobacco extract. Using the rabbit antibody produced, the number and charge of the antigens will be determined by immunoelectrophoresis and crossed-immunoelectrophoresis (13).

B. Analysis of Extracts of Tobacco Smoke

The second step of our investigation will be to prepare a tobacco smoke extract. This will be accomplished by bubbling cigarette smoke through buffered saline at room temperature. The extract will be analyzed as described above for protein content and characteristics of proteins, if any, are present. The smoke extract will be mixed with Freund's complete adjuvant and injected into rabbits in an attempt to stimulate antibody formation. If present, antigens will be characterized using the methods described above for analysis of antigens in the tobacco extract.

C. Analysis of Extracts of Tobacco Smoke Exposed to Human Serum Albumin

The third step in these experiments will be to prepare an extract of cigarette smoke using buffered saline containing human serum albumin. The rationale for this experiment is the possibility that reactive chemicals in smoke may interact with groups on serum albumin, and introduce new antigenic determinants into the albumin molecule. If so the new antigenic determinants may be detected by immunization of rabbits with the smoke-extract-albumin mixture. Accordingly this material will be dialyzed and injected into rabbits. The resulting antiserums will be studied by immunodiffusion and immunoelectrophoresis to determine whether new antigenic determinants are present on the smoke-altered human serum albumin. If antigenic determinants are detected, we will determine whether there are any common antigens among the various extracts. For this study the antiserums to the smoke-extract will be absorbed with human serum albumin to render it specific for the new antigenic determinants. The absorbed serum will then be used in the experiments to determine if common antigens are present.

D. Analysis of Human Serum and Secretions

In the final phase of the experiment we will test human serum and bronchial secretions of smokers collected at the time of bronchoscopy (see attached consent form) for the presence of antibodies against tobacco extract, tobacco smoke extract and tobacco smoke-albumin extract. Immunodiffusion, complement fixation and possibly radioimmunoprecipitation procedures will be used to determine whether such antibodies are present. We recognize that the concentration of antibodies in human serum and secretions may be low. Accordingly we will use radioimmunosorption experiments in which the various smoke derived antigens are insolubilized by reaction with cyanogen-bromide activated microcrystalline cellulose (11). Following reaction of serum or secretions with the solid phase antigen and washing the resulting complex will be exposed to radiolabelled affinity-chromatography-purified antibody to IgA or IgG. This procedure, similar in principle to the radioallergosorbent test (12), is sufficiently sensitive to detect antibodies in the subnanogram (per ml) range. Therefore it should be possible to determine whether antibodies are present in the human specimen.

10. Space and facilities available (when elsewhere than item 2 indicates, state location):

The laboratory facility we have at our disposal, termed the Allergic Diseases Research Laboratory, consists of 1400 sq. ft. of space we alone occupy, and another 500 sq. ft. of shared space and contains all major items of equipment for immunochemical investigations. These include cold laboratories, adequate cold storage and deep freeze space, both -20° and -70°, balances, chromatographic equipment, fluorescent microscopes, ultracentrifuges, both preparative and analytical, polarimeters, equipment for analytical electrophoresis, in all commonly used supporting media, incubators, spectrophotofluorometers and spectrophotometers. Quarters for housing experimental animals, both small animals, such as rabbits and guinea pigs, and large animals, such as goats, horses, and monkeys are available. Special laboratories (hot lab) with remote handling facilities for experiments involving radioactive isotopes, a monitoring system, and dark room facilities are available. We also have an automatic gamma counting system with two channels, an electronic calculator for background subtraction, automatic calculations of counts per minute and ratio computations, and a teletypriter print out. Finally as part of earlier studies on penicillin allergy (92) we have stocks of serum from rabbits immunized with the BPO determinant to aid in experiments with this determinant.

11. Additional facilities required:
NONE

12. Biographical sketches of investigator(s) and other professional personnel (append):

13. Publications: (five most recent and pertinent of investigator(s); append list, and provide reprints if available):

Name: Gerald J. Gleich

Title: Consultant in Microbiology, Mayo Clinic and Mayo Foundation; Associate Professor of Internal Medicine, Immunology, and Microbiology, Mayo Medical School

Date of Birth: REDACTED

Place of Birth: REDACTED REDACTED

Present Nationality:

Sex: Male

Education: Institution	Degree	Year
Michigan College of Mining and Technology University of Michigan University of Michigan Medical School	B.A., M.D.	REDACTED

Honors: Phi Beta Kappa; Phi Kappa Phi; Alpha Omega Alpha; Sigma Xi

Relationship to Proposed Project: Principal Investigator

Research and/or Professional Experience:

Consultant in Microbiology, Mayo Clinic and Mayo Foundation	1965-Present
Associate Professor of Internal Medicine, Immunology and Microbiology, Mayo Medical School	1974-Present
Associate Professor of Internal Medicine and Microbiology, Mayo Medical School	1973-1974
Assistant Professor of Internal Medicine and Microbiology, Mayo Graduate School of Medicine	1968-1973
Instructor in Medicine, Mayo Graduate School of Medicine	1965-1968
Instructor in Medicine and Microbiology, University of Rochester School of Medicine and Dentistry, Rochester, New York	1963-1964
Private Practice of Internal Medicine, Strong Memorial Hospital, Rochester, New York	1963-1964
Assistant Physician, Strong Memorial Hospital, Rochester, New York	1961-1965
Trainee in Allergy and Immunology, University of Rochester Medical Center, Rochester, New York	1961-1963
Residency, Jackson Memorial Hospital, Miami, Florida	1959-1961
Internship, Philadelphia General Hospital, Philadelphia, Pennsylvania	1956-1957

Publications

1. Griffiths, R.W., Gleich, G.J.: Proteolytic Degradation of IgG and Its Relation to Molecular Conformation. J. Biol. Chem. 247:4543-4548, July, 1972.

2. Gleich, G.J., Maldonado, J.E. and Loegering, D.A.: Identification of a Major Basic Protein in Guinea Pig Eosinophil Granules. J. Exp. Med. 137:1459, 1973.

3. Fair, D.S., Gleich, G.J., Krueger, R.G. and Kyle, R.A.: Studies on IgA and IgG Monoclonal Proteins Derived from a Single Patient. I. Evidence for Shared Individually Specific Antigenic Determinants. J. Imm. Vol. 112:201, Jan., 1974.

4. Gleich, G.J., Jones, R.T., Larson, J.B. and Baer, H.: Measurement of the Potency of Allergy Extracts by Their Inhibitory Capacities in the Radioallergosorbent Test. J. Allergy and Clin. Immunology, Vol. 53:148, March, 1974.

5. Gleich, G.J., Loegering, D.A., Kueppers, F., Bajaj, S.P. and Mann, K.G.: Physiochemical and Biological Properties of the Major Basic Protein from Guinea Pig Eosinophil Granules. The Journal of Experimental Medicine, August 1, 1974, Vol. 140, No. 2, pp 313-332.

Name: John C. McDougall, M.D.

Title: Research Fellow, Mayo Clinic and Mayo Foundation

Date of Birth: REDACTED

Place of Birth: REDACTED

Present Nationality: REDACTED

Sex: Male

Education	Institution	Degree	Year
	University of North Dakota	B.S. (Chemistry)	
	University of North Dakota	B.S. (Medicine)	
	Bowman Gray School of Medicine	M.D.	REDACTED

Research and/or Professional Experience

Rotating Internship, Weld County General Hospital	1968-1969
Resident in Internal Medicine, Mayo Graduate School of Medicine	1971-1973
Trainee in Thoracic Disease, Mayo Graduate School of Medicine	1973-Present

14. First year budget:

A. Salaries (give names or state "to be recruited"). Professional (give % time of investigator(s) even if no salary requested)		% time	Amount
G. J. Gleich, M.D.	Prin. Invest.	--	--
J. C. McDougall, M.D.	Co-Investigator	25%	R
Payroll Benefits (15.8%)			R
Technical			
		Sub-Total for A	REDACTED
B. Consumable supplies (by major categories)			
Reagents and Glassware			1,000
Radioisotopes			200
Animals: Purchase (18) and maintenance (6 mo.) of rabbits			1,475
		Sub-Total for B	2,675
C. Other expenses (itemize)			
		Sub-Total for C	--
		Running Total of A + B + C	6,730
D. Permanent equipment (itemize)			
		Sub-Total for D	
E. Indirect costs (15% of A+B+C)		E	1,615
15. Estimated future requirements:		Total request	8,345

	Salaries	Consumable Suppli	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2						
Year 3						

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16. Other sources of financial support:
List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
The Functions of Eosinophils	AI 09728	144,000	5/1/70-4/30/75
Allergic Disease Center Program Grant	AI 11483	109,435	6/1/73-5/31/78
Measurement of the Potency of Allergen Extracts by The Radioallergosorbent Test	FDA 73-164	66,080	4/1/72-3/31/75
Establishment of an Allergen Certification Laboratory	NO1 AI 42546	120,430	6/25/74-6/24/76

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
The Functions of Eosinophils (Renewal)	AI 09728	249,385	5/1/75-4/31/80

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal Investigator

Typed Name G. J. Gleich

Signature Gerald J. Gleich, M.D. Date 11/27/74

Telephone 507 (Area Code) 282-2511 (Number) / 2351 (Extension)

Checks payable to

K. J. Ladner, Mayo Foundation

Mailing address for checks

200 First Street Southwest
Rochester, Minnesota

Responsible officer of institution

Typed Name K. J. Ladner

Title Treasurer

Signature K. J. Ladner Date 11/27/74

Telephone 507 (Area Code) 282-2511 (Number) / 2983 (Extension)

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SIGNIFICANCE

The significance of these studies rests upon the known importance of chronic bronchitis as a cause of morbidity and mortality in the American population. We believe the studies are of theoretical interest in that they will show whether or not there are antigens present in tobacco or tobacco smoke and whether these antigens might incite an immune response in patients. Additional studies which would follow upon a positive result in the initial studies would obviously include the investigation of the effect of these antigens on the lung of experimental animals and their ability to incite inflammatory reactions in the lung as a result of their antigenicity. Were we to identify antigens in tobacco smoke, the characteristics of these materials might suggest ways to rid tobacco of the critical antigens. Alternatively if haptens were to be found we would investigate their ability to provoke inflammation in the lung and investigate ways in which these might be removed from tobacco smoke. Finally, if indeed immunologic reactions to tobacco antigens were shown, it might be possible to utilize the antibodies produced by the host as an index of the extent or severity of the inflammation occuring in the lungs, and thus as a diagnostic test of the potential severity of the pathologic process. This would provide us with a new diagnostic tool for the identification of individuals at greatest risk from inhalation of tobacco smoke.

DESCRIPTION FOR LAY INFORMATION

A series of experiments will be performed to determine whether or not tobacco smoke when inhaled into the lungs may cause disease of the lungs by stimulating an allergic or immunologic response to the tobacco smoke. Studies of a preliminary nature will be performed using animal subjects. If these are promising, studies of human blood and bronchial secretions will also be performed to seek clues as to how the body's own defense mechanism might be turned against itself to produce chronic bronchitis and emphysema, two major health problems in America today.

BIBLIOGRAPHY

1. Green, Gareth M.: Lung defense mechanisms. Med. Clin. N. A. 57:547-562, 1973.

2. United States Department of Health, Education and Welfare. The Health Consequences of Smoking: A report of the Surgeon General, 1972. DHEW Publication No. HSM 72-7516) Washington, D.C., Government Printing Office, 1972, Chapter 3.

3. Anderson, A.E., Jr., Hernandez, J.A., Holmes, W.L. et al: Pulmonary Emphysema: prevalence, severity and anatomical patterns in macrosections with respect to smoking habits. Arch. Environ. Health 12:569-577, 1966.

4. Petty, T.L., Ryan, S.F., Mitchell, R.S.: Cigarette smoking and the lungs: relation to post-mortem evidence of emphysema, chronic bronchitis and black lung pigmentation. Arch. Environ. Health 14:172-177, 1967.

5. Auerbach, O., Hammond, E.C., Garfinkel, L., et al: Relation of smoking and age to emphysema: whole lung section study. N. Eng. J. Med. 286:853-857, 1972.

6. Spain, D.M., Siegel, H., Bradess, V.A.: Emphysema in apparently healthy adults: smoking, age and sex. JAMA 224:322-325, 1973.

7. Pipes, D.M.: Allergy to tobacco smoke. Ann. of All. 3:277-282, 1945.

8. Harkavy, J., Witebsky, E.: Studies of specificity in multiple hypersensitiveness by quantitative titration and absorption of reagins. J. of All. 6:437-447, 1934-1935.

9. Peshkin, N.M., Landy, L.H.: Cutaneous reactions to tobacco antigen in allergic and nonallergic children with the direct and indirect (local passive transfer) methods of testing. The J. of All. 10:241-245, 1938-1939.

10. Silvette, H., Larson, P.S., Haag, H.B.: Immunological aspects of tobacco and smoking. Am. J. of the Med. Sci. 233:561-589, November 1957.

11. Yunginger, J. W., Gleich, G.J.: Comparison of the protein-binding capacities of cyanogen bromide-activated polysaccharides. The J. of Allergy and Clin. Immunol., Vol. 50, No. 2 109-116, 1972.

12. Yunginger, J.W., Gleich, G.J.: Seasonal Changes in IgE Antibodies and Their Relationship to IgG Antibodies during Immunotherapy for Ragweed Hay Fever. 52: 1268-1275, May, 1973.

13. Ganrot, P.O.: Crossed Immunoelectrophoresis. Scand. J. Clin. Lab. Invest. 29:39-41, 1972. Suppl 124.

CONSENT

_____, the undersigned is willing to participate in a study of lung disease, under the direction of Dr. G. J. Gleich and associates, Mayo Clinic and Mayo Foundation.

I acknowledge that the following has been discussed with me in detail:

1. That as a result of my participation in this study fifteen milliliters of whole blood (about one-half ounce) will be taken from me.

2. That the removal of blood involves inserting a needle into a vein and that this may be uncomfortable.

3. That a sample of my bronchial secretions (phlegm) will be taken during the bronchoscopy procedure which I will have performed for other medical reasons.

4. That the collection of bronchial secretions involves no added risk since they are normally removed and discarded during bronchoscopy.

5. That this study has as its goal the uncovering of new information which may help in the understanding of the relationship between tobacco smoking and some forms of lung disease. This information is not likely to benefit me directly, though it conceivably might.

6. That any inquiries I have concerning this study have been answered, and I have been informed that I am free to withdraw my consent and to discontinue participation in this study, project or activity at any time, without prejudice to my care.

_____
Signed

_____
Investigator

_____
Date

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